**PCT** WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/66 — A1

(11) International Publication Number: WO 97/04785

(43) International Publication Date: 13 February 1997 (13.02.97)

(21) International Application Number: PCT/EP96/03301

(22) International Filing Date: 26 July 1996 (26.07.96)

(30) Priority Data:
2213/95-3 28 July 1995 (28.07.95) CH

(71) Applicant *(for all designated States except US)*: SYMPHAR S.A. [CH/CH]; 243, route des Fayards, CH-1290 Versoix (CH).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: NIESOR, Eric [FR/CH]; 13C, chemin de Bonmont, CH-1260 Nyon (CH). BENTZEN, Craig, Leigh [US/CH]; 3, chemin de la Chevrerie, Pre-Bonnet, CH-1279 Bogis-Bossey (CH). NGUYEN, Lan, Mong [CH/CH]; 14, la Levratte, CH-1260 Nyon (CH). THUILLARD, Jean-Luc [CH/CH]; 660, Champ-de-Joux, CH-1264 Saint-Cergue (CH). PHAN, Hieu, Trung [CH/CH]; 8, chemin de Benuyer, CH-1295 Tannay (CH). FLACH, Jean [CH/CH]; 38, avenue d'Echallens, CH-1004 Lausanne (CH).

(74) Agents: HUTCHINS, Michael, Richard et al.; Fry Heath & Spence, The Old College, 53 High Street, Horley, Surrey RH6 7BN (GB).

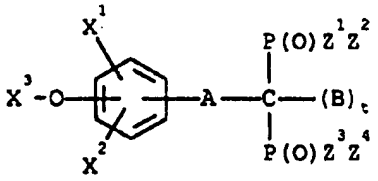
(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

**Published**

*With international search report.*

*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: USE OF PHENOL SUBSTITUTED DIPHOSPHONATES AS ANTINEOPLASTIC AGENTS




(I)

(57) Abstract

The invention provides the use of a phenol substituted gem-diphosphonate selected from the compounds of formula (I), where $Z^1$, $Z^2$, $Z^3$ and $Z^4$ are identical or different and are OR where R is H, a straight, branched or cyclic alkyl group comprising from 1 to 8 carbon atoms, OM where M is a cation, $NR_2$ where R has the same meaning as defined above, $Z^1$, $Z^2$ and $Z^3$, $z^4$ may form an alkylidenedioxy ring comprising from 2 to 8 carbon atoms, $X^1$, $X^2$ are identical or different and are H, a halogen atom, a straight, branched or cyclic alkyl or alkoxy group from 1 to 8 carbon atoms, $X^3$ is H, an alkyl group $R^1$ from 1 to 4 carbon atoms, an acyl group $C(O)R^1$, a carbamyl group $C(O)NHR^1$ where $R^1$ is described as above, $X^3O$ and one of the two other substituents $X^1$ or $X^2$ may form an alkylidenedioxy ring comprising from 1 to 4 carbon atoms, A is $-CH=CH-CH_2-$, $-(CH_2)_n-$, $-O(CH_2)_n-$, -S-, $-SO_{¿}2-_{¿}$, $-S(CH_2)_n-$, $-SO_2(CH_2)_n-$, where n is an integer from 1 to 7 or together with B forms an alkylidene group of the fromula $-(CH=CH)_k-(CH_2)_d-CH=$ where k is zero or 1 and d is an integer from zero to 4, B is H, an alkyl group from 1 to 4 carbon atoms, t is zero or 1, with the proviso that t is zero only when A is $(CH=CH)_k-(CH_2)_d-CH=$ where k and d are as described above, for the manufacture of a medicament for the treatment or prevention of neoplastic diseases and in particular ras dependent cancers.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | |
|---|---|
| **AM** | Armenia |
| **AT** | Austria |
| **AU** | Australia |
| **BB** | Barbados |
| **BE** | Belgium |
| **BF** | Burkina Faso |
| **BG** | Bulgaria |
| **BJ** | Benin |
| **BR** | Brazil |
| **BY** | Belarus |
| **CA** | Canada |
| **CF** | Central African Republic |
| **CG** | Congo |
| **CH** | Switzerland |
| **CI** | Côte d'Ivoire |
| **CM** | Cameroon |
| **CN** | China |
| **CS** | Czechoslovakia |
| **CZ** | Czech Republic |
| **DE** | Germany |
| **DK** | Denmark |
| **EE** | Estonia |
| **ES** | Spain |
| **FI** | Finland |
| **FR** | France |
| **GA** | Gabon |
| **GB** | United Kingdom |
| **GE** | Georgia |
| **GN** | Guinea |
| **GR** | Greece |
| **HU** | Hungary |
| **IE** | Ireland |
| **IT** | Italy |
| **JP** | Japan |
| **KE** | Kenya |
| **KG** | Kyrgystan |
| **KP** | Democratic People's Republic of Korea |
| **KR** | Republic of Korea |
| **KZ** | Kazakhstan |
| **LI** | Liechtenstein |
| **LK** | Sri Lanka |
| **LR** | Liberia |
| **LT** | Lithuania |
| **LU** | Luxembourg |
| **LV** | Latvia |
| **MC** | Monaco |
| **MD** | Republic of Moldova |
| **MG** | Madagascar |
| **ML** | Mali |
| **MN** | Mongolia |
| **MR** | Mauritania |
| **MW** | Malawi |
| **MX** | Mexico |
| **NE** | Niger |
| **NL** | Netherlands |
| **NO** | Norway |
| **NZ** | New Zealand |
| **PL** | Poland |
| **PT** | Portugal |
| **RO** | Romania |
| **RU** | Russian Federation |
| **SD** | Sudan |
| **SE** | Sweden |
| **SG** | Singapore |
| **SI** | Slovenia |
| **SK** | Slovakia |
| **SN** | Senegal |
| **SZ** | Swaziland |
| **TD** | Chad |
| **TG** | Togo |
| **TJ** | Tajikistan |
| **TT** | Trinidad and Tobago |
| **UA** | Ukraine |
| **UG** | Uganda |
| **US** | United States of America |
| **UZ** | Uzbekistan |
| **VN** | Viet Nam |

# USE OF PHENOL SUBSTITUTED DIPHOSPHONATES AS ANTINEOPLASTIC AGENTS

The present invention relates to antineoplastic agents, and in particular to the use of phenol substituted gem-diphosphonate derivatives in the treatment of neoplastic diseases. More specifically, the present invention provides the use of certain phenol substituted gem-diphosphonate derivatives for the preparation of pharmaceutical compositions useful in the treatment and prevention of cancers and metastasis and in particular useful in the treatment and prevention of ras oncogene dependent cancers and metastatic invasions.

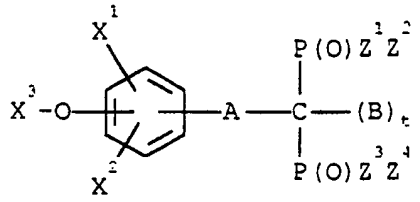
The majority of existing anticancer drugs are cytotoxic compounds which lack specificity for killing tumor cells and therefore also affect normal cells, resulting in toxic side effects. There remains therefore a need for the development of more specific agents acting on the cell signalling pathways leading to the inhibition of cancer cell proliferation without affecting normal cell proliferation (Oncolytic Drugs,J.R.Prous,The Year's Drug News,1994 edition, p.459 and Ras Oncogene Directed Approaches in Cancer Chemotherapy ,G.Bolton et. al., Annual Reports in Medicinal Chemistry 1994; 29: 165-174).

Mutations of the ras oncogene have been shown to be present in a wide variety of human tumors and may contribute to as many as one-fifth of all human cancers. Specifically, it is found in more than fifty percent of colon and ninety percent of pancreatic carcinomas. Ras mutations are therefore considered to play a key role in triggering cancer formation and development (J. L. Bos, Cancer Res. 1989;49:4682-4689). It has also been established that the mutated forms of ras protein are present only in tumors and not in normal tissues of cancer patients. Blocking the activity of ras mutations to transform normal cells into cancer cells as well as to further promote the development of cancer cells and tumors is therefore an attractive therapeutic target.

The US patent N° 5,043,330 (1991) corresponding to the European Patent N° 0,339,237 discloses the synthesis of a class of phenol substituted gem-diphosphonate derivatives and their utility as lipid lowering agents, for example in the treatment of cardiovascular diseases.

The present applicants have now found that diphosphonates of the type disclosed in US 5,043,330 are surprisingly effective for inhibiting specifically the proliferation as well as inducing apoptosis in cancer cells without being cytotoxic to normal cells.

Accordingly, in one aspect, the invention provides the use of a compound for the manufacture of a medicament for the treatment of neoplastic diseases, said compound having the following formula (I):



(I)

where :

- $Z^1$, $Z^2$, $Z^3$ and $Z^4$ are identical or different and are
  - OR where R is H, a straight, branched or cyclic alkyl group comprising from 1 to 8 carbon atoms,
  - OM where M is a cation,
  - $NR_2$ where R has the same meaning as defined above,
  - $Z^1$, $Z^2$ and $Z^3$, $Z^4$ may form an alkylidenedioxy ring comprising 2 to 8 carbon atoms,
- $X^1$and $X^2$ are identical or different and are H, a halogen atom, a straight, branched or cyclic alkyl or alkoxy group from 1 to 8 carbon atoms,

- $X^3$ is H, an alkyl group $R^1$ from 1 to 4 carbon atoms, an acyl group $C(O)R^1$, a carbamyl group $C(O)NHR^1$ where $R^1$ is described as above, $X^3O$ and one of the two other substituents $X^1$ or $X^2$ may form an alkylidenedioxy ring comprising from 1 to 4 carbon atoms,

- A is $-CH=CH-CH_2-$, $-(CH_2)_n-$, $-O(CH_2)_n-$, -S-, $-SO_2-$, $-S(CH_2)_n-$, $-SO_2(CH_2)_n$, where n is an integer from 1 to 7, or together with B forms an alkylidene group of the formula $(CH=CH)_k-(CH_2)_d-CH=$ where k is zero or 1 and d is an integer from zero to 4,

- B is H, an alkyl group from 1 to 4 carbon atoms,

- t is zero or 1, with the proviso that t is zero only when A is $(CH=CH)_k-(CH_2)_d-CH=$ where k and d are as described above.

The compounds of formula (I) can exist as salts and references to the compounds of formula (I) hereinafter include the salt forms of the compounds, unless the context indicates otherwise. Examples of salts are compounds of formula (I) wherein one or more of the groups $Z^1$, $Z^2$, $Z^3$ and $Z^4$ are constituted by the group OM where M is an alkaline or alkaline earth metal ion or an ammonium group $NR_4$ where R has the same meaning as defined above.

In another aspect the invention provides the use of a compound of the formula (I) as hereinbefore defined for the manufacture of a medicament for treating solid tumors, for example colon, pancreas, thyroid, lung, breast, head and neck tumors.

In another aspect the invention provides the use of a compound of the formula (I) as hereinbefore defined for the manufacture of a medicament for treating tumors of the hemopoietic and immune system, for example lymphomas and leukemias.

In still another aspect the invention provides the use of a compound of the formula (I) as hereinbefore defined for the manufacture of a medicament for treating patients with metastasis of primary tumors.

In a further aspect, the invention provides the use of a compound of formula (I) as hereinbefore defined for the manufacture of a medicament for preventing the transformation of normal cells or inhibiting metastatic invasion of normal tissues by cancer cells.

In a still further aspect, the invention provides a method of treatment of neoplastic diseases or prevention of cancer metastasis, and in particular ras-dependent cancers, which method comprises administering to a patient suffering from said cancer or the potential of cancer development, an effective therapeutic amount of a compound of the formula (I) as hereinbefore defined.
The invention also includes within its scope a method for selectively eradicating cancer cells which comprises treating a mixture of cancer cells and normal cells from a patient in an *ex vivo* manner with a compound of the formula (I), further to which the cells are reintroduced into the patient. Thus, for example, in accordance with this method, blood, plasma or other fluids can be drawn off from the patient, treated with the compounds of formula (I) *ex vivo*, and then reintroduced into the patient.

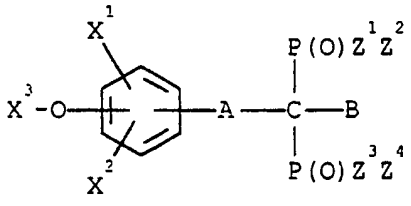
In the compounds of formula (I), examples of groups $Z^1$, $Z^2$, $Z^3$ and $Z^4$ include hydroxy, methoxy, ethoxy, n-propyloxy, isopropyloxy, n-butyloxy, sec-butyloxy and tert-butyloxy. A preferred group is the isopropyloxy group.

It is presently preferred that the groups $Z^1$, $Z^2$, $Z^3$ and $Z^4$ are identical and in a particularly preferred embodiment of the invention $Z^1$, $Z^2$, $Z^3$ and $Z^4$ are all isopropyloxy.

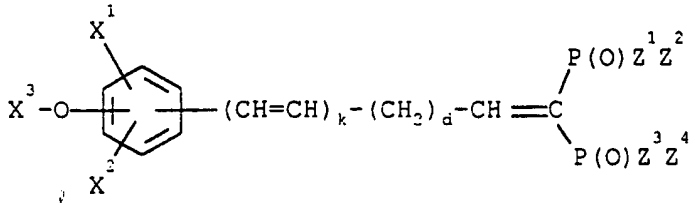
Examples of groups $X^1$ and $X^2$ include hydrogen, straight or branched alkyl groups and alkoxy groups having from 1 to 5 carbon atoms, more particularly from 1 to 4 carbon atoms. Preferred groups $X^1$ and $X^2$ are methyl, ethyl, n-propyl, isopropyl, sec-butyl, tert-butyl, methoxy and ethoxy groups, a particularly preferred group being tert-butyl.

Examples of groups $X^3$ include hydrogen, $C_{1-4}$ alkyl and $C_{1-4}$ alkanoyl, hydrogen being particularly preferred at present.

The compounds of formula (I) include the phenol substituted alkylidenediphosphonates (Ia) and the phenol substituted alkenylidenediphosphonates (Ib).



(Ia)



(Ib)

where $X^1$, $X^2$, $X^3$, A, B, k, d, $Z^1$, $Z^2$, $Z^3$ and $Z^4$ are as described above.

Compounds of structure (Ia) include, for example, those in which :

- $X^1$ and $X^2$ are identical or different and are alkyl groups from 1 to 8 carbon atoms,
- $X^3$ is hydrogen,

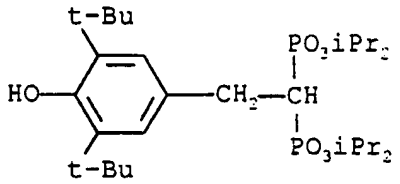
- A is CH=CH-$CH_2$, $(CH_2)_n$, S, $SO_2$, S-$(CH_2)_n$, $SO_2$-$(CH_2)_n$, where n is 1-7,
- B is hydrogen or a $C_1$-$C_4$ alkyl group,
- $Z^1$, $Z^2$, $Z^3$ and $Z^4$ are identical or different and are OH, alkoxy groups of 1 to 8 carbon atoms or one or both of the pairs $Z^1$, $Z^2$ and $Z^3$, $Z^4$ are an alkylidenedioxy group of 2 to 8 carbon atoms.

Compounds of structure (Ib) include, for example, those in which

- $X^1$ and $X^2$ are identical or different and are alkyl groups from 1 to 8 carbon atoms,
- $X^3$ is hydrogen,
- k is zero or 1 and d is zero to 4,
- $Z^1$, $Z^2$, $Z^3$, $Z^4$ identical or different are OH, alkoxy groups of 1 to 8 carbon atoms or one or both of the pairs $Z^1$, $Z^2$ and $Z^3$, $Z^4$ are an alkylidenedioxy group of 2 to 8 carbon atoms.

Particular examples of compounds of formula (I) for use in the present invention include the compounds in Tables 1a and 1b.

This invention provides a new use of gem-diphosphonates of formula (I) for the treatment of neoplastic diseases and prevention of cancers and metastasis, and more particularly those which are ras dependent. In a particular preferred embodiment, it provides a new use of Compound 1 of formula (I), wherein $X^1$ and $X^2$ are both tert-butyl respectively at the 3- and 5- positions, $X^3$ is H at the 4-position, A is $CH_2$, B is H, t is 1 and $Z^1$, $Z^2$, $Z^3$, $Z^4$ all are iso-propyloxy for the preparation of pharmaceutical compositions useful for the treatment of cancers. Said Compound 1 has the following structure, formula and physicochemical properties:




Tetraisopropyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate, $C_{28}H_{52}O_7P_2$, mp = 104-105°C

The compounds of the invention can be prepared according to the methods described in EP 0 339 237 A, the disclosure of which is incorporated by reference herein, or by methods analogous thereto.

Some of the analogs are novel. Accordingly, in a further aspect, the invention provides a novel compound selected from:

tetraisopropyl 2-(3,5-diisopropyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraisopropyl 2-(3,5-diisopropyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate,

tetraisopropyl 2-(3,4,5-trimethoxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraisopropyl 2-(3,4,5-trimethoxyphenyl)-ethylidene-1,1-diphosphonate,

tetraisopropyl 2-(3-tert-butyl-4-hydroxy-5-methylphenyl)-ethenylidene-1,1-diphosphonate,

tetraisopropyl 2-(3-tert-butyl-4-hydroxy-5-methylphenyl)-ethylidene-1,1-diphosphonate,

tetraisopropyl 2-(3-ethoxy-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraisopropyl 2-(3-ethoxy-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate,

tetraethyl 2-(3,5-di-tert-butyl-4-methoxyphenyl)-ethenylidene-1,1-diphosphonate and

tetraisopropyl 1-(3,5-di-tert-butyl-4-hydroxyphenyl)butylidene-2,2-diphosphonate.

The compounds of formula (I) can be administered orally, or by delivery across another mucosal surface (for example across the nasal, buccal, bronchial or rectal mucosa), transdermally, or by injection (for example intradermal, intraperitoneal, intravenous or intramuscular injection).

When the compounds are intended for oral administration, they can be formulated, for example, as tablets, capsules, granules, pills, dragees, lozenges, powders, solutions, emulsions, syrups, suspensions, or any other pharmaceutical form suitable for oral administration. Oral dosage forms can, if desired, be coated with one or more release delaying coatings to allow the release of the active compound to be controlled or targeted at a particular part of the enteric tract.

Tablets and other solid or liquid oral dosage forms can be prepared in standard fashion from the compounds of formula (I) and a pharmaceutically acceptable solubilizer, diluent or carrier. Examples of solubilizers, diluents or carriers include sugars such as lactose, starches, cellulose and its derivatives, powdered tracaganth,

malt, gelatin, talc, stearic acid, magnesium stearate, calcium sulfate, vegetable oils, polyols such as glycerol, propyleneglycol and polyethyleneglycols, alginic acids and alginates, agar, pyrogen free water, isotonic saline, phosphate buffered solutions, and optionally other pharmaceutical excipients such as disintegrants, lubricants, wetting agents such as sodium lauryl sulfate, coloring agents, flavoring agents and preservatives, etc..

Capsules can be of the hard or soft variety and can contain the active compound in solid, liquid or semisolid form. Typically such capsules are formed from gelatine or an equivalent substance and can be coated or uncoated. If it is desired to delay the release of the active compound until the capsule has passed through the stomach and into the intestine, the capsule can be provided with a pH sensitive coating adapted to dissolve at the pH found in the duodenum or ileum. Examples of such coatings include the Eudragits, the uses of which are well known.

Formulations for injection will usually be made up of the appropriate solubilizers such as detergents which may also include compounds and excipients such as buffering agents to provide an isotonic solution having the correct physiological pH. The injectable solutions are typically pyrogen-free and can be provided in sealed vials or ampoules containing a unit dose of compound.

A unit dosage form of the compounds of the invention typically will contain from 0.1% to 99% by weight of the active substance, more usually from 5% to 75% of the active substance. By way of example, a unit dosage form can contain from 1mg to 1g of the compound, more usually from 10mg to 500mg, for example between 50mg and 400mg, and typically in doses of 100mg to 200mg.

The compounds of the invention will be administered in amounts which are effective to provide the desired therapeutic effect. The concentrations necessary to provide the desired therapeutic effect will vary according to among other things the precise nature of the disease, the size, weight and age of the patient and the severity of the disease.

The doses administered will preferably be non-toxic to the patient, although in certain circumstances the severity of the disease under treatment may necessitate administering an amount of compound which causes some signs of toxicity.

Typically, the compounds of the invention will be administered in amounts in the range 0.01 mg/kg to100 mg/kg bodyweight, more preferably 0.1 mg/kg to 10 mg/kg bodyweight and particularly 1 mg/kg to 5 mg/kg bodyweight. For an average human of 70 kg weight, a typical daily dosage of the compounds of the invention would be in the range of 70 mg to 700 mg. Such a dosage can be administered, for example from two to four times daily. Ultimately however, the size of the doses administered and the frequency of administration will be at the discretion and judgement of the physician treating the patient.

Example K is provided to illustrate a representative batch formula used by the applicants to prepare capsules of Compound 1.

The pharmacological activity of the compounds of the present invention can be demonstrated by means of an *in vitro* screening model using a clone of NIH 3T3 cells transfected with the human bladder cancer T24 (H-ras) oncogene. This cell line (PAP2) has been selected based upon its characterisation of a high level of ras expression and ras-dependent activities which correlate with the high level of metastatic ability (S.A. Hill et al., in J. of the National Cancer Institute_ 1988; 80: 484-490 and A. Chambers et. al. in Invasion and Metastasis 1990; 10: 225-240). The PAP2 cell line has been shown to have a ras-dependent increased expression of cathepsins, cysteine proteinases implicated in the processes of metastasis (A. Chambers et al., in Molecular Carcinogenesis 1992; 5:238-245). Thus PAP2 cells exhibit functions which are relevant to the pathogenesis of human cancers. These cells were used for testing *in vitro* the effect of compounds on cell proliferation, proteolytic enzyme activity (metastasis) and apoptosis (programmed cell death). When injected s.c. in immunodeficient (nude) mice these cells rapidly form solid tumours and the anticancer activity of the tested compounds were measured *in vivo* after oral administration.

The results of a series of *in vitro* and *in vivo* tests led to the discovery by the applicants that representative compounds of formula (I) and in particular Compound 1,

- inhibit the growth of cancer cells in tissue culture,
- induce apoptosis in cancer cells in tissue culture,
- inhibit proteases in cancer cells which are involved in metastasis, and
- demonstrate anti-cancer activity in nude mice bearing solid tumors.

The experimental results presented in Tables 1-7b provide evidence that compounds of formula (I), and in particular Compound 1, are potentially useful in the treatment of neoplastic diseases which include cancers of the hemopoietic and immune system, such as lymphomas and leukemias, as well as cancers of the pancreas, colon, breast, thyroid, brain, lung, head and neck. This recently discovered anticancer activity of compounds of formula (I) is unexpected and is independent of their previously reported activities as lipid lowering agents.

Results are expressed as mean ± sem. Significance of difference was estimated using the Student *t*-test for unpaired data.

**Example 1**

**INHIBITION OF RAS-DEPENDENT CELLULAR PROLIFERATION BY COMPOUNDS OF FORMULA (I)**

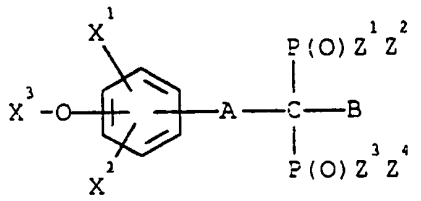
A series of compounds of formula (I) were screened to determine the most active compounds and structure activity relationships. The inhibition of PAP2 cell proliferation was selected as an initial screening test.

Briefly, PAP2 cells were seeded at a concentration of $3x10^4$ per well in 24-well plates and were allowed to attach for 24h. Test compounds were added at 10 and 20µM final concentrations in 1% ethanol solutions. Cells were trypsinized after 48 h incubation and viable cells (excluding Trypan blue) were counted. The results obtained are listed in Tables 1a and 1b for the series of Compounds (Ia) and (Ib) respectively.

The compounds screened in this test were synthesized according to the procedures described in the US patent 5,043,330 (1991) corresponding to the European patent 0 339 237. Some examples (Examples A-J) are provided to further illustrate the synthesis of novel derivatives according to the procedures described in the above-mentioned prior art documents.

## Table 1a

### Effect of phenol substituted gem-diphosphonates (Ia) on PAP2 Cells

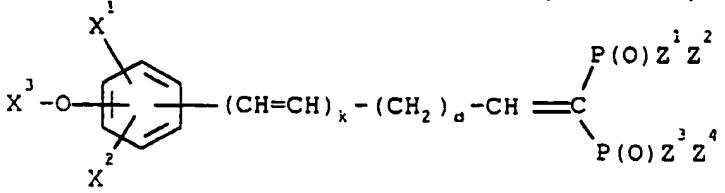



(Ia)

| Cpd | $X^1$ | $X^2$ | $X^3$ | A | B | $Z^1$ | $Z^2$ | $Z^3$ | $Z^4$ | Cell (% count control) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 10μM | 20μM |
| 1 | 3-t-Bu | 5-t-Bu | 4-H | $CH_2$ | H | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -70 | -100 |
| 2 | 3-t-Bu | 5-t-Bu | 4-H | $CH_2$ | H | OH | OH | OH | OH | -31 | -45 |
| 3 | 3-t-Bu | 5-t-Bu | 4-H | $CH_2$ | H | OMe | OMe | OMe | OMe | -21 | -17 |
| 4 | 3-t-Bu | 5-t-Bu | 4-H | $CH_2$ | H | OEt | OEt | OEt | OEt | -30 | -22 |
| 5 | 3-t-Bu | 5-t-Bu | 4-H | $CH_2$ | H | On-Pr | On-Pr | On-Pr | On-Pr | -22 | -89 |
| 6 | 3-t-Bu | 5-t-Bu | 4-H | $CH_2$ | H | On-Bu | On-Bu | On-Bu | On-Bu | -26 | -51 |
| 7 | 3-s-Bu | 5-s-Bu | 4-H | $CH_2$ | H | OEt | OEt | OEt | OEt | -6 | +3 |
| 8 | 3-s-Bu | 5-s-Bu | 4-H | $CH_2$ | H | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -21 | -45 |
| 9 | 3-i-Pr | 5-i-Pr | 4-H | $CH_2$ | H | OEt | OEt | OEt | OEt | -16 | -31 |
| 10 | 3-i-Pr | 5-i-Pr | 4-H | $CH_2$ | H | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -20 | -51 |
| 11 | 3-t-Bu | 5-Me | 4-H | $CH_2$ | H | OEt | OEt | OEt | OEt | -14 | -37 |
| 12 | 3-t-Bu | 5-Me | 4-H | $CH_2$ | H | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -3 | -27 |
| 13 | 3-t-Bu | 5-t-Bu | 4-H | S | H | OEt | OEt | OEt | OEt | -19 | -39 |
| 14 | 3-t-Bu | 5-t-Bu | 4-H | S | H | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -53 | -90 |
| 15 | 3-OMe | 5-OMe | 4-Me | $CH_2$ | H | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -23 | -5 |
| 16 | 3-OEt | 5-H | 4-H | $CH_2$ | H | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -2 | +5 |
| 17 | 3-t-Bu | 5-t-Bu | 4-H | $CH_2$ | H | OEt | OEt | On-Bu | On-Bu | -6 | -95 |
| 18 | 3-t-Bu | 5-t-Bu | 4-H | $CH_2$ | H | OEt | OEt | Oi-Pr | Oi-Pr | -46 | -59 |
| 19 | 3-t-Bu | 5-t-Bu | 4-H | $CH_2$ | Et | OEt | OEt | OEt | OEt | -21 | -36 |
| 20 | 6-Cl | 3,4-O | $CH_2$ | $CH_2$ | H | OEt | OEt | OEt | OEt | +20 | -9 |
| 21 | 3-t-Bu | 5-t-Bu | 4-H | $CH_2$ | H | $O(CH_2)_3O$ | | $O(CH_2)_3O$ | | -32 | -28 |
| 22 | 3-OMe | 5-OMe | 4-H | $CH_2$ | H | OEt | OEt | OEt | OEt | | |
| 23 | 3-OMe | 5-OMe | 4-H | $CH_2$ | H | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | | |
| 24 | 3-t-Bu | 5-t-Bu | 4-H | $CH_2$ | Et | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -25 | -40 |
| | | | | | | | | | | | |
| | | | | | | | | | | | |

## Table 1b

### Effect of phenol substituted gem-diphosphonates (Ib) on PAP2 Cells




(Ib)

| Cpd | $X^1$ | $X^2$ | $X^3$ | k | d | $Z^1$ | $Z^2$ | $Z^3$ | $Z^4$ | Cell count (% control) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 10μM | 20μM |
| 25 | 3-t-Bu | 5-t-Bu | 4-H | 0 | 0 | OH | OH | OH | OH | | |
| 26 | 3-t-Bu | 5-t-Bu | 4-H | 0 | 0 | OMe | OMe | OMe | OMe | -33 | -17 |
| 27 | 3-t-Bu | 5-t-Bu | 4-H | 0 | 0 | OEt | OEt | OEt | OEt | -48 | -45 |
| 28 | 3-t-Bu | 5-t-Bu | 4-H | 0 | 0 | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -28 | -46 |
| 29 | 3-t-Bu | 5-t-Bu | 4-H | 0 | 0 | On-Pr | On-Pr | On-Pr | On-Pr | -51 | -63 |
| 30 | 3-t-Bu | 5-t-Bu | 4-H | 0 | 0 | On-Bu | On-Bu | On-Bu | On-Bu | -13 | -31 |
| 31 | 3-s-Bu | 5-s-Bu | 4-H | 0 | 0 | OEt | OEt | OEt | OEt | -30 | -44 |
| 32 | 3-s-Bu | 5-s-Bu | 4-H | 0 | 0 | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -13 | -54 |
| 33 | 3-i-Pr | 5-i-Pr | 4-H | 0 | 0 | OEt | OEt | OEt | OEt | -5 | +13 |
| 34 | 3-i-Pr | 5-i-Pr | 4-H | 0 | 0 | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -26 | -62 |
| 35 | 3-t-Bu | 5-Me | 4-H | 0 | 0 | OEt | OEt | OEt | OEt | -14 | -14 |
| 36 | 3-t-Bu | 5-Me | 4-H | 0 | 0 | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -24 | -7 |
| 37 | 3-OMe | 5-OMe | 4-H | 0 | 0 | OEt | OEt | OEt | OEt | -3 | -28 |
| 38 | 3-OMe | 5-OMe | 4-H | 0 | 0 | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | | |
| 39 | 3-OMe | 5-OMe | 4-Me | 0 | 0 | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -17 | -34 |
| 40 | 3-OEt | 5-H | 4-H | 0 | 0 | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -30 | -23 |
| 41 | 3-t-Bu | 5-t-Bu | 4-H | 1 | 0 | OEt | OEt | OEt | OEt | -40 | -77 |
| 42 | 3-t-Bu | 5-t-Bu | 4-H | 1 | 0 | Oi-Pr | Oi-Pr | Oi-Pr | Oi-Pr | -36 | -71 |
| 43 | 3-t-Bu | 5-t-Bu | 4-H | 0 | 0 | OEt | OEt | On-Bu | On-Bu | -18 | -54 |
| 44 | 3-t-Bu | 5-t-Bu | 4-H | 0 | 0 | OEt | OEt | Oi-Pr | Oi-Pr | -17 | -28 |
| 45 | 3-t-Bu | 5-t-Bu | 4-Me | 0 | 0 | OEt | OEt | OEt | OEt | -10 | -33 |
| 46 | H | 3,4- | $OCH_2$ | 0 | 0 | OEt | OEt | OEt | OEt | -22 | -26 |
| 47 | H | 3,4- | $(OCH_2)_2$ | 0 | 0 | OEt | OEt | OEt | OEt | -11 | -30 |
| | | | | | | | | | | | |
| | | | | | | | | | | | |

## Example 2

## *In vitro* Results

## INHIBITION OF RAS-DEPENDENT CELLULAR PROLIFERATION

### Cell culture

H-Ras-transfected NIH 3T3 cells (PAP2) (S.A. Hill et al.; J Natl. Cancer Inst. 1988;80:484-490) were grown at 37°C in a 5% $CO_2$ atmosphere in Dulbecco's modified Eagle medium (DMEM) with 25mM HEPES and 10% fetal calf serum. PAP2 cells were trypsinized and subcultured twice a week prior confluency.

### 1. Inhibition of Cell proliferation

The effects of Compound 1 on cellular proliferation were monitored by two methods:

- cell count with a hemacytometer and concurrent DNA determination
- cell number estimation by colorimetric assay.

#### 1.1. Cell count and DNA content

Briefly, PAP2 cells were seeded at a concentration of $3x10^4$ per dish in 24-well plates 4h before the addition of increasing concentrations of the tested compound. Cells were trypsinized at daily intervals. An aliquot of the cell suspension was counted with a hemacytometer. The remaining cells were lysed in 0.01N NaOH and DNA concentration was determined by spectrofluorimetry using 4,6-diamidino-2-phenylindole as fluorochrome and calf thymus DNA as standard.

**Table 2a**

**Inhibition of the Proliferation of PAP2 Cells by Compound 1**

(cell number/well)

| | Compound 1 concentration | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.1µM | 0.5µM | 1.0µM | 5.0µM | 10µM |
| Cell nb/well | 273750 | 256250 | 208750 | 186250 | 102500 | 71250 |
| sem | 9437 | 7465 | 4270 | 15861 | 5951 | 8260 |
| % change | | -6 | -24 | -32 | -63 | -74 |
| p | | 0.196 | 0.001 | 0.003 | 0.001 | 0.001 |

The calculated IC50 value of Compound 1 for the inhibition of the growth of PAP2 cells is 1.02µM.

**Table 2b**

**Decrease in DNA Concentration Produced by Compound 1 in Cultured PAP2 Cells**

DNA (mg/well)

| | Compound 1 concentration | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.1µM | 0.5µM | 1.0µM | 5.0µM | 10µM |
| DNA (mg/well) | 4.62 | 4.40 | 4.04 | 1.99 | 1.66 | 0.34 |
| sem | 0.14 | 0.18 | 0.42 | 0.14 | 0.07 | 0.04 |
| % change | | -5 | -13 | -57 | -64 | -93 |
| p | | 0.375 | 0.240 | 0.001 | 0.001 | 0.001 |

The calculated IC50 value of Compound 1 for decreasing the DNA content of PAP2 cells is 2.77µM.

The results in Tables 2a and 2b show that compounds of formula (I), in particular Compound 1, inhibit the growth of PAP2 cells in culture.

1.2. Colorimetric MTT assay

Cell number was estimated by the MTT assay performed essentially as described by T. Mosmann in J. Immunol Method 1983;65:55-63.
Briefly, PAP2 cells were seeded at a concentration of $1x10^4$ per dish in flat 96-well plates (Falcon) 5h prior the addition of the tested drugs. Following an incubation period of 24h, 48h or 72h, 10µl of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] dissolved in PBS at 5mg/ml were added to each well and incubated at 37°C for 4h. The medium was then removed and 100µl of 0.04N HCl in isopropanol were added to the wells. Absorbance of the converted dye was measured at 570nm by a microplate reader with background substraction at 620nm.

The calculated IC50 value of Compound 1 for the inhibition of the proliferation of PAP2 cells is 8.05µM, as measured by the MTT method (after 72h incubation). The results in Tables 3a, 3b and 3c confirm by another assay that compounds of formula (I), in particular Compound 1, inhibit the growth of PAP2 cells in culture.

**Effect of Compound 1 on the Proliferation of PAP2 Cells Measured by the MTT assay**

**Table 3a**
**Incubation with Compound 1 for 24h**
(OD at 570nm)

| | Compound 1 concentration | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1.0µM | 2.5µM | 5µM | 10µM | 25µM |
| OD | 39.9 | 39.5 | 37.6 | 35.9 | 28.9 | 21.4 |
| sem | 2.1 | 4.6 | 1.2 | 1.0 | 1.1 | 1.0 |
| % change | 0 | -1 | -6 | -10 | -28 | -46 |
| p | | 0.942 | 0.370 | 0.109 | 0.001 | 0.001 |

**Table 3b**
**Incubation with Compound 1 for 48h**
(OD at 570nm)

| | Compound 1 concentration | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1.0µM | 2.5µM | 5µM | 10µM | 25µM |
| Mean | 71.3 | 66.5 | 59.4 | 52.8 | 37.3 | 15.1 |
| sem | 5.2 | 4.9 | 2.5 | 3.1 | 1.9 | 0.7 |
| % change | 0 | -7 | -17 | -26 | -48 | -79 |
| p | | 0.516 | 0.059 | 0.009 | 0.001 | 0.001 |

**Table 3c**
**Incubation with Compound 1 for 72h**
(OD at 570nm)

| | Compound 1 concentration | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1.0µM | 2.5µM | 5µM | 10µM | 25µM |
| Mean | 366.0 | 336.8 | 334.4 | 287.4 | 136.6 | 29.0 |
| sem | 30.3 | 19.0 | 18.6 | 16.5 | 15.5 | 6.3 |
| % change | | -8 | -9 | -21 | -63 | -92 |
| p | | 0.427 | 0.388 | 0.039 | 0.001 | 0.001 |

**3. Inhibition of DNA synthesis**

DNA synthesis was measured by the incorporation of tritiated thymidine into trichloroacetic acid (TCA) precipitable material by cultured PAP2 cells.
PAP2 cells were seeded in 24-well plates at $3x10^4$ cells per well for 2 days. Then following preincubation with Compound 1 dissolved in ethanol, 0.5µCi of methyl-[3H]-thymidine (specific activity 82 Ci/mmol) were added and labelling proceeded for 4 hours. The cells were then washed with 1ml of cold phosphate-buffered saline (PBS), solubilized with 0.2ml of 4% sodium dodecyl sulfate. The cellular extract was precipitated with 1ml of 30% TCA, and kept for 1h on ice. The precipitates were collected by filtration onto fiber glass filters and washed with 5ml of 5% TCA. Radioactivity on the filters was counted in a liquid scintillation counter.

**Table 4**
**Inhibition of DNA Synthesis by Compound 1**
**in ras Transfected Cells (PAP2)**

3H-Thymidine incorporation into DNA
(cpm)

| | Compound 1 concentration | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.5μM | 2μM | 5μM | 10μM | 25μM |
| cpm | 53021 | 45607 | 37130 | 30226 | 18772 | 14100 |
| sem | 7296 | 5354 | 2590 | 3945 | 1481 | 2766 |
| % change | 0 | -14 | -30 | -43 | -65 | -73 |
| p | | 0.427 | 0.075 | 0.016 | 0.009 | 0.005 |

The calculated IC50 value of Compound 1 for the inhibition DNA synthesis in PAP2 cells is 3.75μM.

Results in Table 4 show that compounds of formula (I), in particular Compound 1, inhibit DNA synthesis in ras transfected cells.

## 4. Induction of apoptosis

Compounds of formula (I) have also been shown to have a selective effect in stimulating cell death in the PAP2 cell line at 5uM or higher concentrations. This specific effect in selectively stimulating programmed cell death in cancer cells was verified in two other human cancer cell lines (HepG2 and SW480) with Compound 1 indicating that these compounds not only inhibit cell growth but in addition produce cancer cell self-destruction (Table 5). This has been confirmed by gel electrophoresis showing DNA fragmentation, the hallmark of apoptosis.

**Table 5**

**The Effect of Compound 1 on Cell Viability in Human Cancer Cell Lines**

| Cell line | Nb of Viable Cells at 0 hrs | Nb of Viable Cells at 24 hrs | % Change |
|---|---|---|---|
| SW 480 | 38'800 | 0 | -100.0% |
| HepG2 | 214'700 | 82'000 | -61.8% |

- Cells are cultured in 24-well plates for 48h prior treatment.
- Treatments are 10µM compounds for 24h.
- Counting : cells are trypsinized and viable cells are counted.

## 5. Effect of Compound 1 on a wide range of human cancer cell lines

Compound 1 was tested for its anti-proliferative activity in a wide range of established human derived cancer cell lines to confirm the potential use of these compounds in relevant human cancers (Table 6). The results indicate that Compound 1 is effective in decreasing cell proliferation in greater than 90% of the more than 50 human cancer cell lines tested. Compounds of formula (I) therefore can be considered useful in treating a wide range of human cancers with and without ras-mutations.

**Table 6**

**Anti-Proliferative Effects Compound 1 on Human Tumor Derived Cell Lines**

| Tumor Type | Cell line | Inhibition of Proliferation (% Control) |
|---|---|---|
| Leukemia | CCRF-CEM | -61.8 |
| Leukemia | HL-60 (tb) | -95.3 |
| Leukemia | K-562 | -61.2 |
| Non-Small Cell Lung Cancer | A549/ATCC | -55.0 |
| Non-Small Cell Lung Cancer | EKVX | -27.0 |
| Non-Small Cell Lung Cancer | HOP-62 | -13.8 |
| Non-Small Cell Lung Cancer | HOP-92 | -85.1 |
| Non-Small Cell Lung Cancer | NCI-H23 | -21.8 |
| Non-Small Cell Lung Cancer | NCI-H322M | -12.5 |
| Non-Small Cell Lung Cancer | NCI-H460 | -40.7 |
| Non-Small Cell Lung Cancer | NCI-H522 | -59.8 |
| Colon Cancer | COLO 205 | -55.9 |
| Colon Cancer | HCC-2998 | -50.7 |
| Colon Cancer | HCT-116 | -68.7 |
| Colon Cancer | HCT-15 | -19.5 |
| Colon Cancer | HT29 | -83.0 |
| Colon Cancer | KM12 | -32.5 |
| Colon Cancer | SW-620 | -21.4 |
| CNS Cancer | SF-268 | -21.2 |
| CNS Cancer | SF-295 | -49.6 |
| CNS Cancer | SF-539 | +1.5 |
| CNS Cancer | SNB-19 | -55.8 |
| CNS Cancer | SNB-75 | -54.4 |
| CNS Cancer | U251 | -41.6 |
| Melanoma | LOX IMVI | -18.7 |
| Melanoma | MALME-3M | -20.4 |
| Melanoma | M14 | -66.7 |
| Melanoma | SK-MEL-2 | -6.1 |
| Melanoma | SK-MEL-28 | -25.0 |
| Melanoma | SK-MEL-5 | -51.8 |
| Melanoma | UACC-257 | -26.9 |

| | | |
|---|---|---|
| Melanoma | UACC-62 | -30.9 |
| Ovarian Cancer | IGROV1 | -24.4 |
| Ovarian Cancer | OVCAR-3 | -47.3 |
| Ovarian Cancer | OVCAR-4 | -18.9 |
| Ovarian Cancer | OVCAR-5 | -17.6 |
| Ovarian Cancer | OVCAR-8 | -37.6 |
| Ovarian Cancer | SK-OV-3 | -28.1 |
| Renal Cancer | 786-0 | -28.7 |
| Renal Cancer | ACHN | -43.4 |
| Renal Cancer | CAKI-1 | -31.1 |
| Renal Cancer | SN12C | -1.0 |
| Renal Cancer | TK-10 | -18.6 |
| Renal Cancer | UO-31 | -35.9 |
| Prostate Cancer | PC-3 | -75.9 |
| Prostate Cancer | DU-145 | -21.3 |
| Breast Cancer | MCF-7 | -57.0 |
| Breast Cancer | MCF-7/ADR-RES | -49.3 |
| Breast Cancer | MDA-MB-231/ATCC | -50.9 |
| Breast Cancer | MDA-MB-435 | -51.6 |
| Breast Cancer | MDA-N | -43.3 |
| Breast Cancer | BT-549 | -51.3 |
| Breast Cancer | T-47D | -62.1 |

## Example 2

### *In vivo* Results

### INHIBITION OF TUMOUR GROWTH

Seven to nine-week old female nude mice (Swiss nu/nu) were injected s.c. with 0.5ml of PAP2 cells ($5x10^5$ cells per 0.5ml of DMEM) on day 0. Oral treatment with Compound 1 (50mg/kg) started on the same day. The treated group (n=18) received Compound 1 mixed with food (0.03% w/w) and the control group (n=22) received a diet without added compound. At day 15, the mice were weighed, sacrificed and the tumors were excised and weighed.

**Table 7a**
**Effect of Oral Treatment with Compound 1 (50mg/kg) on Tumor Weight Produced by s.c. Injection of PAP2 cells in Nude Mice**

| Animal Group | Body Weight (g) (mean ± sem) | Tumor Weight (g) (mean ± sem) |
|---|---|---|
| Control (n = 21) | 24.2 ± 0.5 | 0.228 ± 0.041 |
| Treated (n = 18) | 24.6 ± 0.7 | 0.053 ± 0.016 |
| % change | +2 | -77 |
| p | 0.6470 | 0.0006 |

The results in Table 7a show that the mean tumor weight in the treatment group decreased significantly, indicating that Compound 1 is a potent antitumor agent. Mice body weight in both control and treated groups are identical, thus establishing the lack of toxicity of Compound 1.

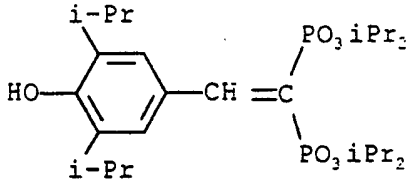
In another set of experiments Compound 1 was tested at doses of 12.5, 50 and 100mg/kg. The results in Table 7b show that Compound 1 inhibits tumor growth in nude mice at doses as low as 12.5mg/kg and in a dose dependent manner. These results demonstrate that compounds of formula (I), in particular Compound 1, are potent orally active antitumor compounds which do not have a toxic effect on normal cells, tissues or organs.

**Table 7b**
**Decrease in Tumour Weight by Oral Treatment of Nude Mice Injected with PAP2 Cells with Different Doses of Compound 1**

| Animal Group | Body Weight (g) (mean ± sem) | Tumor Weight (g) (mean ± sem) |
|---|---|---|
| Control (n=14) | 24.7 ± 0.6 | 0.276 ± 0.056 |
| Treated, 12.5 mg/kg (n=5) | 23.9 ± 0.8 | 0.053 ± 0.026 |
| % change | -3 | -81 |
| p | 0.4562 | 0.0472 |
| Treated, 50 mg/kg (n=6) | 27.2 ± 1.0 | 0.037 ± 0.020 |
| % change | +10 | -87 |
| p | 0.0523 | 0.0211 |
| Treated, 100 mg/kg (n=6) | 24.5 ± 0.3 | 0.025 ± 0.011 |
| % change | -1 | -91 |
| p | 0.7619 | 0.0151 |

Example A

Tetraisopropyl 2-(3,5-diisopropyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate




Titanium tetrachloride (13.83g, 73 mmol) was added dropwise to dry THF (80 ml) maintained at 0°C. The resulting mixture was treated sequentially at 0°C with 3,5-diisopropyl-4-hydroxybenzaldehyde (5.0g, 24 mmol), tetraisopropyl methylenediphosphonate (10.85g, 32 mmol) and N-methylmorpholine (14.71g, 146 mmol). The reaction mixture was stirred for 12 h at room temperature and 80 ml water were added. The quenched reaction mixture was extracted with diethylether (3 X 60ml), the combined ether fractions were extracted with a saturated NaCl solution until the aqueous washes had a neutral pH. After drying over $MgSO_4$, the organic solvent was evaporated and the residue was purified by column chromatography on silica using as eluant a mixture of $CH_2Cl_2$:MeOH (95:5) to give 8g (62%) of a solid; mp=87-88°C.

MS : m/e = 532 : $M^+$, 367 : $M^+ - PO_3iPr_2$

NMR ($CDCl_3$)

$\delta$ = 8.22 (dd, J= 31 and 48 Hz, 1H) : Ph-C<u>H</u>=C-$P_2$

7.7 (s, 2H) : aromatic H,

5.6 (s, 1H) : OH,

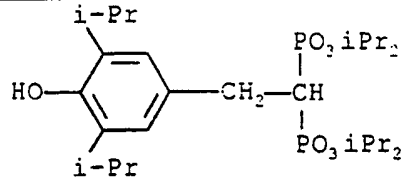
4.85-4.63 (2m, 4H) : P-O-C<u>H</u>$Me_2$

3.16 (septet, 2H): Ph-C<u>H</u>$Me_2$

1.39, 1.36, 1.27, 1.23 and 1.16 (8d, 36H total): P-O-CH<u>Me</u>$_2$ and Ph-CH<u>Me</u>$_2$

Example B

Tetraisopropyl 2-(3,5-diisopropyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate



Tetraisopropyl 2-(3,5-diisopropyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate (5g, 9.4 mmol) was dissolved in ethanol (50ml) and the solution was hydrogenated for 4h over 2g of 10% palladium on carbon at 50 psi at room temperature. The catalyst was filtered, the solvent was evaporated and the residue was purified by column chromatography on silica using as eluant a mixture of $CH_2Cl_2$:MeOH (95:5) to give 2.5g (50%) of a solid; mp=90-91°C

MS : m/e = 534 : $M^+$, 369 : $M^+ - PO_3iPr_2$

NMR ($CDCl_3$)

δ = 6.94 (m, 2H) : aromatic H,

4.8-4.7 (m, 5H) : P-O-C<u>H</u>$Me_2$ and OH

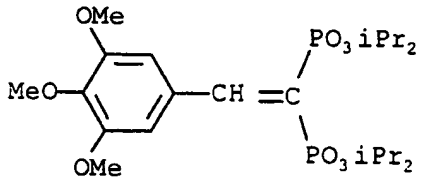
3.2-3.1 (several m, 6H total ) : Ph-C<u>H</u>$_2$-CH and Ph-C<u>H</u>$Me_2$

2.51 (tt, J = 6 and 24 H, 1H) : Ph-$CH_2$-C<u>H</u>

1.33-1.21 (several d, 36H total): P-O-CH<u>Me</u>$_2$ and Ph-CH<u>Me</u>$_2$

<u>Example C</u>

<u>Tetraisopropyl 2-(3,4,5-trimethoxyphenyl)-ethenylidene-1,1-diphosphonate</u>




3,4,5-Trimethoxybenzaldehyde (7g, 35.7mmol) was treated with titanium tetrachloride, tetraisopropyl methylenediphosphonate and N-methylmorpholine in THF as described in Example A to give 11.5g (62%) of the title compound.

MS : m/e = 522 : $M^+$, 357 : $M^+ - PO_3iPr_2$

NMR ($CDCl_3$)

δ = 8.21 (dd, J= 30 and 48 Hz, 1H) : Ph-C<u>H</u>=C-$P_2$

7.28 : (s, 2H) : aromatic H

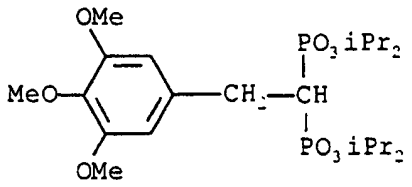
4.85-4.63 (2m, 4H) : P-O-C<u>H</u>$Me_2$

3.9 (t, 9H) : Ph-O<u>Me</u>

1.4, 1.36 and 1.22 (4d, 24H total): P-O-CH<u>Me</u>$_2$

<u>Example D</u>

Tetraisopropyl 2-(3,4,5-trimethoxyphenyl)-ethylidene-1,1-diphosphonate



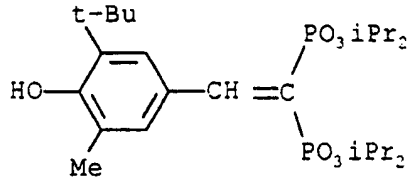
Tetraisopropyl 2-(3,4,5-trimethoxyphenyl)-ethenylidene-1,1-diphosphonate (7g, 13.4 mmol) was hydrogenated over 10% Pd/C as described in Example B to give 5.7g (81%) of the title compound.

MS : m/e = 524 : $M^+$, 359 : $M^+$ - $PO_3iPr_2$

NMR ($CDCl_3$)
δ = 6.55 (s, 2H) : aromatic H,
4.8-4.7 (m, 4H) : P-O-C<u>H</u>$Me_2$
3.85 and 3.81 (2s, 9H) : Ph-O<u>Me</u>
3.17 (dt, J = 6 and 16 Hz, 2H) : Ph-C<u>$H_2$</u>-CH
2.50 (tt, J= 6 and 24 Hz, 1H) : Ph-$CH_2$-C<u>H</u>
1.33-1.26 (several d, 24H total): P-O-CH<u>$Me_2$</u>

Example E

Tetraisopropyl 2-(3-tert-butyl-4-hydroxy-5-methylphenyl)-ethenylidene-1,1-diphosphonate



3-Tert-butyl-4-hydroxy-5-methylbenzaldehyde (6g, 31.3mmol) was treated with titanium tetrachloride, tetraisopropyl methylenediphosphonate and N-methylmorpholine in THF as described in Example A to give 4.2g (62%) of the title compound.

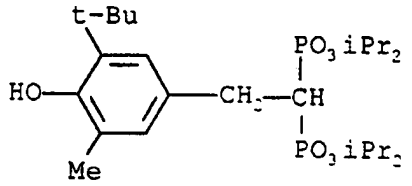
MS : m/e = 518 : $M^+$, 353 : $M^+$ - $PO_3iPr_2$

NMR ($CDCl_3$)
δ = 8.19 (dd, J= 30 and 48 Hz, 1H) : Ph-C<u>H</u>=C-$P_2$

7.71-7.67 (m, 2H) aromatic H,
5.6 (s, 1H) : OH,
4.8-4.7 (2m, 4H): P-O-C<u>H</u>$Me_2$
2.26 (s, 3H) : Ph-<u>Me</u>
1.40 (s, 9H) : Ph-t-<u>Bu</u>
1.38, 1.36, 1.24 and 1.20 (8d, 24H total): P-O-CH<u>$Me_2$</u>

<u>Example F</u>

<u>Tetraisopropyl 2-(3-tert-butyl-4-hydroxy-5-methylphenyl)-ethylidene-1,1-diphosphonate</u>



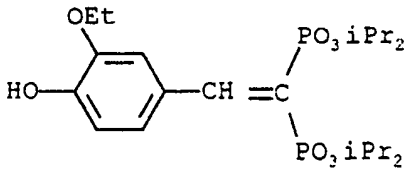

Tetraisopropyl 2-(3-tert-butyl-4-hydroxy-5-methylphenyl)-ethenylidene-1,1-diphosphonate (5g, 9.6 mmol) was hydrogenated over 10% Pd/C as described in Example B to give 2.9g (58%) of the title compound.

MS : m/e = 520 : $M^+$, 355 : $M^+$ - $PO_3iPr_2$

NMR ($CDCl_3$)
δ = 7.02 and 6.92 (2m, 2H) : aromatic H,
4.8-4.7 (m, 5H) : P-O-C<u>H</u>$Me_2$ and OH
3.11 (dt, J = 6 and 17 Hz, 2H) : Ph-C<u>$H_2$</u>-CH
2.49 (tt, J= 6 and 24 Hz, 1H) : Ph-$CH_2$-C<u>H</u>
2.21 (s, 3H) : Ph-<u>Me</u>
1.39 (s, 9H) : Ph-t-<u>Bu</u>
1.31, 1.26 and 1.24( 3d, 24H): P-O-CH<u>$Me_2$</u>

<u>Example G</u>

<u>Tetraisopropyl 2-(3-ethoxy-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate</u>

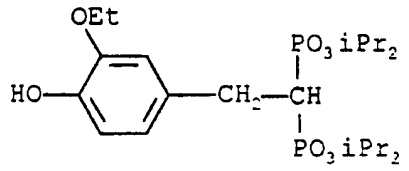


3-Ethoxy-4-hydroxybenzaldehyde (6g, 36.1mmol) was treated with titanium tetrachloride, tetraisopropyl methylenediphosphonate and N-methylmorpholine in THF as described in Example A to give 4.2g (62%) of the title compound, mp = 143-144°C.

MS : m/e = 492 : $M^+$, 327 : $M^+$ - $PO_3iPr_2$

NMR ($CDCl_3$)
δ = 8.19(dd, J= 31 and 48 Hz, 1H) : Ph-C<u>H</u>=C-$P_2$
7.9, 7.3 and 6.91(3m, 3H) : aromatic H,
6.2 (s, 1H) : OH
4.85-4.63 (2m, 4H) : P-O-C<u>H</u>$Me_2$
4.19 (q, J = 7Hz) : Ph-OC<u>$H_2$</u>-$CH_3$
1.46 (t, J = 7Hz) : Ph-$OCH_2$-C<u>$H_3$</u>
1.39, 1.36, 1.23 and 1.21( 4d, 24 H total) : Ph-CH<u>Me</u>$_2$

<u>Example H</u>
<u>Tetraisopropyl 2-(3-ethoxy-4-hydroxy-phenyl)-ethylidene-1,1-diphosphonate</u>



Tetraisopropyl 2-(3-ethoxy-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate (5g, 10.16 mmol) was hydrogenated over 10% Pd/C as described in Example B to give 4.5g (90%) of the title compound.

MS : m/e = 494 : $M^+$, 329 : $M^+$ - $PO_3iPr_2$

NMR ($CDCl_3$)
δ = 6.85-6.75 (several m, 3H) : aromatic H

5.65 (s, 1H) : OH

4.8-4.7 (m, 4H) : P-O-C<u>H</u>$Me_2$

4.10 (q, J = 7Hz) : Ph-O$C\underline{H}_2$-$CH_3$

3.14 (dt, J = 6 and 16 Hz, 2H) : Ph-$C\underline{H}_2$-CH

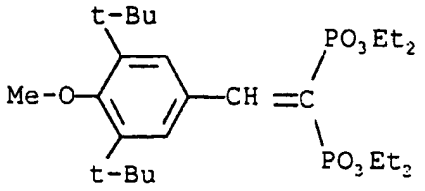
2.45 (tt, J= 6 and 24 Hz, 1H) : Ph-$CH_2$-C<u>H</u>

1.43 (t, J = 7Hz) : Ph-O$CH_2$-$C\underline{H}_3$

1.32-1.25 (4 partially overlapping d, 24H total): P-O-CH<u>$Me_2$</u>

<u>Example I</u>

<u>Tetraethyl 2-(3.5-di-tert-butyl-4-methoxyphenyl)-ethenylidene-1.1-diphosphonate</u>




3,5-Di-tert-butyl-4-methoxybenzaldehyde (2.0g, 8.1 mmol) was treated with titanium tetrachloride, tetraethyl methylenediphosphonate and N-methylmorpholine in THF as described in Example A to give 3.0g (72%) of the title compound, mp=66-67°C.

MS : m/e = 518 : $M^+$, 381: $M^+$ - $PO_3Et_2$

NMR ($CDCl_3$)

δ = 8.26 (dd, J= 30 and 48 Hz, 1H) : Ph-C<u>H</u>=C-$P_2$

7.8 (s, 2H) : aromatic H,

4.25-4.00 (2m, 8H) : P-O-$C\underline{H}_2$-$CH_3$

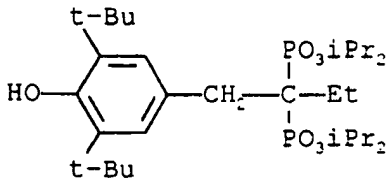
3.69 (s, 3H) : Ph-O<u>Me</u>

1.44 (s,18H) : Ph-t-<u>Bu</u>

1.38 and 1.70 ( 2t, 12 H) : Ph-O$CH_2$-$C\underline{H}_3$

<u>Example J</u>

<u>Tetraisopropyl 1-(3.5-di-tert-butyl-4-hydroxyphenyl)-butylidene-2.2-diphosphonate</u>



Tetraisopropyl propylidene-1,1-diphosphonate was prepared by reacting tetraisopropyl methylenediphosphonate with 3 equivalents of ethyl iodide in presence of NaH in THF.

Tetraisopropyl propylidene-1,1-diphosphonate (2.2g, 6.0 mmol) was added to a suspension of 60% NaH (0.5g, 12.0 mmol) in 20ml of dry THF and the mixture was stirred until the NaH disappeared. 3,5-di-tert-butyl-4-hydroxybenzylchloride (1,5g, 6 mmol) in 10ml THF was added and the mixture was refluxed overnight. After work up, column chromatography on silica using $CHCl_3$ : AcOEt (8:2) as eluant gave 1.5g (42%) of the title compound.

MS : m/e = 590 : $M^+$, 425 : $M^+ - PO_3{}^iPr_2$, base Peak 341: $M^+$ - 2 x propen

mp= 131-132°C

Example K

Typical Example of Compound Formulation

Active Component

Compound 1

Inactive Component

Pregelatinazed Starch NF

Size 3 opaque dark blue gelatin capsules

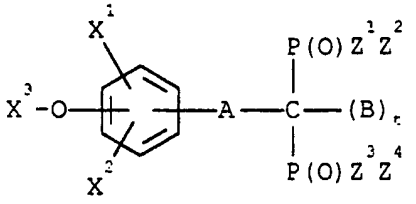
Representative Batch Formula

Typical batch size 2000 capsules

| Ingredients | g/batch | | |
|---|---|---|---|
| | 1mg | 10mg | 50mg |
| Compound 1 | 2.00 | 20.0 | 100.0 |
| Pregelatinized starch NF | 431.6 | 408.8 | 291.0 |
| Total | 433.6 | 428.8 | 391.0 |

CLAIMS

1. Use of a phenol substituted gem-diphosphonate selected from the compounds of formula (I) :



(I)

where :

- $Z^1$, $Z^2$, $Z^3$ and $Z^4$ are identical or different and are
- OR where R is H, a straight, branched or cyclic alkyl group comprising from 1 to 8 carbon atoms,
- OM where M is a cation,
- $NR_2$ where R has the same meaning as defined above,
- $Z^1$, $Z^2$ and $Z^3$, $Z^4$ may form an alkylidenedioxy ring comprising from 2 to 8 carbon atoms,

- $X^1$, $X^2$ are identical or different and are H, a halogen atom, a straight, branched or cyclic alkyl or alkoxy group from 1 to 8 carbon atoms,

- $X^3$ is H, an alkyl group $R^1$ from 1 to 4 carbon atoms, an acyl group $C(O)R^1$, a carbamyl group $C(O)NHR^1$ where $R^1$ is described as above, $X^3O$ and one of the two other substituents $X^1$ or $X^2$ may form an alkylidenedioxy ring comprising from 1 to 4 carbon atoms,

- A is $-CH=CH-CH_2-$, $-(CH_2)_n-$, $-O(CH_2)_n-$, $-S-$, $-SO_2-$, $-S(CH_2)_n-$, $-SO_2(CH_2)_n-$, where n is an integer from 1 to 7 or together with B forms an alkylidene group of the formula $-(CH=CH)_k-(CH_2)_d-CH=$ where k is zero or 1 and d is an integer from zero to 4,

- B is H, an alkyl group from 1 to 4 carbon atoms,

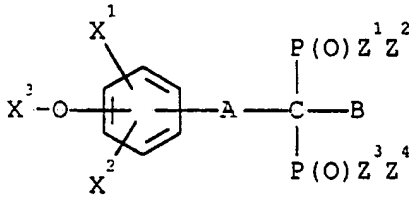
- t is zero or 1, with the proviso that t is zero only when A is $(CH=CH)_k-(CH_2)_d-CH=$ where k and d are as described above,

for the manufacture of a medicament for the treatment or prevention of neoplastic diseases.

2. Use according to claim 1 wherein the neoplastic disease is selected from cancers of the hemopoietic and immune system which comprise lymphomas and leukemias, as well as cancers of the pancreas, colon, breast, thyroid, brain, lung, head and neck.

3. Use according to claim 1 of a diphosphonate selected among compounds of formula (I) for the manufacture of a medicament for the prevention of metastasic invasion of normal tissues by cancer cells.

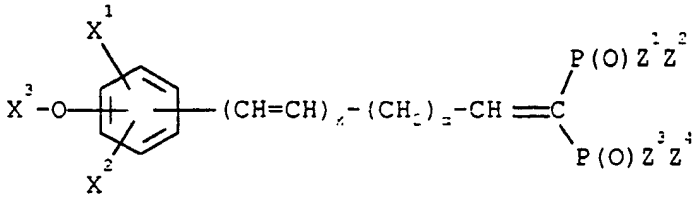
4. Use according to any one of the claims 1 to 3 of a phenol substituted alkylidene diphosphonate selected among compounds of formula (Ia)



(Ia)

where $X^1$, $X^2$, $X^3$, A, B, $Z^1$, $Z^2$, $Z^3$ and $Z^4$ are as defined in claim 1.

5. Use according to any one of the claims 1 to 3 of a phenol substituted alkenylidene diphosphonate selected among compounds of formula (Ib)



(Ib)

where $X^1$, $X^2$, $X^3$, k, d, $Z^1$, $Z^2$, $Z^3$ and $Z^4$ are as defined in claim 1.

6. Use according to any one of the preceding claims wherein $Z^1$, $Z^2$, $Z^3$ and $Z^4$ are the same or different and are selected from methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl and t-butyl.

7. Use according to claim 6 wherein $Z^1$, $Z^2$, $Z^3$ and $Z^4$ are identical.

8. Use according to claim 7 wherein $Z^1$, $Z^2$, $Z^3$ and $Z^4$ are isopropyl groups.

9. Use according to any one of the preceding claims wherein the groups $X^1$ and $X^2$ are the same or different and are selected from straight or branched chain alkyl groups and alkoxy groups having from 1 to 5 carbon atoms.

10. Use according to claim 9 wherein $X^1$ and $X^2$ are the same or different and are selected from methyl, ethyl, n-propyl, isopropyl, s-butyl, t-butyl, methoxy and ethoxy groups.

11. Use according to claims 10 wherein $X^1$ and $X^2$ are the same.

12. Use according to claim 11 wherein $X^1$ and $X^2$ are both t-butyl.

13. Use according to any one of the preceding claims wherein $X^3$ is selected from hydrogen, $C_{1-4}$alkyl and $C_{1-4}$ alkanoyl.

14. Use according to claim 13 wherein $X^3$ is hydrogen.

15. Use according to any one of claims 1 to 3 wherein the compound of the formula (I) is tetraisopropyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate.

16. Use according to any one of claims 1 to 3 of a phenol substituted gem-diphosphonate selected among:

2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonic acid
tetramethyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate
tetraethyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate,
tetra-n-propyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate,
tetra-n-butyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate,
tetraisopropyl 2-(3,5-di-sec-butyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate,

tetraethyl 2-(3,5-diisopropyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate,

tetraisopropyl 2-(3,5-diisopropyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate,

tetraethyl 2-(3-tert-butyl-4-hydroxy-5-methylphenyl)-ethylidene-1,1-diphosphonate,

tetraisopropyl 2-(3-tert-butyl-4-hydroxy-5-methylphenyl)-ethylidene-1,1-diphosphonate,

tetraethyl 3,5-di-tert-butyl-4-hydroxyphenyl thiomethylene-diphosphonate,

tetraisopropyl 3,5-di-tert-butyl-4-hydroxyphenyl thiomethylene-diphosphonate,

tetraisopropyl 2-(3,4,5-trimethoxyphenyl)-ethylidene-1,1-diphosphonate,

dibutyl diethyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate,

diethyl diisopropyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate,

tetraethyl 1-(3,5-di-tert-butyl-4-hydroxyphenyl)-butylidene-2,2-diphosphonate,

2-(3,5-di-tert-butyl-4-hydroxyphenyl)ethylidene-1,1-bis(2-oxo-1,3,2-dioxaphosphorinan),

tetraisopropyl 1-(3,5-di-tert-butyl-4-hydroxyphenyl)-butylidene-2,2-diphosphonate,

2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonic acid,

tetramethyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraethyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraisopropyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetra-n-propyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetra-n-butyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraethyl 2-(3,5-di-sec-butyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraisopropyl 2-(3,5-di-sec-butyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraisopropyl 2-(3,5-diisopropyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraethyl 2-(3-tert-butyl-4-hydroxy-5-methylphenyl)-ethenylidene-1,1-diphosphonate,

tetraisopropyl 2-(3-tert-butyl-4-hydroxy-5-methylphenyl)-ethenylidene-1,1-diphosphonate,

tetraethyl 2-(3,5-dimethoxy-4-hydroxyphenyl)-ethenylidene-1,1- diphosphonate,

tetraisopropyl 2-(3,5-dimethoxy-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraisopropyl 2-(3,4,5-trimethoxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraisopropyl 2-(3-ethoxy-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraethyl 4-(3,5-di-tert-butyl-4-hydroxyphenyl)-1,3-butadienylidene-1,1-diphosphonate,

tetraisopropyl 4-(3,5-di-tert-butyl-4-hydroxyphenyl)-1,3-butadienylidene-1,1-diphosphonate,

dibutyl diethyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

diethyl diisopropyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraethyl 2-(3,5-di-tert-butyl-4-methoxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraethyl 2-(3,4-methylenedioxyphenyl)-ethenylidene-1,1-diphosphonate and

tetraethyl 2-(3,4-ethylenedioxyphenyl)-ethenylidene-1,1-diphosphonate.

17. A method of treating or preventing a neoplastic disease in a patient, which method comprises administering an effective therapeutic amount of a compound of the formula (I) as defined in any one of the preceding claims.

18. A method according to claim 17 wherein the neoplastic disease is selected from cancers of the hemopoietic and immune system which comprise lymphomas and leukemias, as well as cancers of the pancreas, colon, breast, thyroid, brain, lung, head and neck.

19. A method according to claim 17 or claim 18 wherein the cancer is a ras dependent cancer.

20. A method of preventing or inhibiting metastatic invasion of normal tissues by cancer cells in a patient, which method comprises administering to the patient an effective amount of a compound of the formula (I) as defined in any one of the preceding claims to inhibit metastatic processes.

21. A method of preventing the transformation of a normal cell into a tumor cell induced by the mutated ras activities, which method comprises treating the cell with an amount of a compound of the formula (I), as defined in any one of the preceding claims, effective to block the ras activities.

22. A method according to claim 21 wherein the normal cell is present in a patient.

23. A method for selectively eradicating cancer cells which comprises treating a mixture of cancer cells and normal cells from a patient in an *ex vivo* manner with a compound of the formula (I), further to which the cells are reintroduced into the patient.

24. A method according to claim 23 wherein the cells are reintroduced into the patient by injection or infusion.

25. A compound selected from the group consisting of:

tetraisopropyl 2-(3,5-diisopropyl-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraisopropyl 2-(3,5-diisopropyl-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate,

tetraisopropyl 2-(3,4,5-trimethoxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraisopropyl 2-(3,4,5-trimethoxyphenyl)-ethylidene-1,1-diphosphonate,

tetraisopropyl 2-(3-tert-butyl-4-hydroxy-5-methylphenyl)-ethenylidene-1,1-diphosphonate,

tetraisopropyl 2-(3-tert-butyl-4-hydroxy-5-methylphenyl)-ethylidene-1,1-diphosphonate,

tetraisopropyl 2-(3-ethoxy-4-hydroxyphenyl)-ethenylidene-1,1-diphosphonate,

tetraisopropyl 2-(3-ethoxy-4-hydroxyphenyl)-ethylidene-1,1-diphosphonate,

tetraethyl 2-(3,5-di-tert-butyl-4-methoxyphenyl)-ethenylidene-1,1-diphosphonate and

tetraisopropyl 1-(3,5-di-tert-butyl-4-hydroxyphenyl)butylidene-2,2-diphosphonate.

26. A pharmaceutical composition comprising a compound as defined in claim 25 and a pharmaceutically acceptable carrier.

27. A compound according to claim 25 for use in treatment of cancers, in particular ras dependent cancers.

# INTERNATIONAL SEARCH REPORT

Inte tional Application No
PCT/EP 96/03301

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/66

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | US,A,5 043 330 (NGUYEN ET AL) 27 August 1991 cited in the application --- | |
| A | EP,A,0 440 809 (TORAY INDUSTRIES) 14 August 1991 ----- | |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 13 December 1996 | 20.12.96 |
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax (+31-70) 340-3016 | Authorized officer<br>Klaver, T |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 96/03301

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
In view of the large number of compounds which are defined by the wording of the claims, the search has been performed on the general idea and compounds mentioned in the examples of the description.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 96/03301

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
|---|---|---|---|---|
| US-A-5043330 | 27-08-91 | CH-A- | 675422 | 28-09-90 |
| | | AU-A- | 3224389 | 05-10-89 |
| | | CA-A- | 1338495 | 30-07-96 |
| | | CN-A,B | 1036772 | 01-11-89 |
| | | CN-A- | 1086820 | 18-05-94 |
| | | EP-A- | 0339237 | 02-11-89 |
| | | ES-T- | 2011210 | 01-01-94 |
| | | FI-B- | 92933 | 14-10-94 |
| | | IE-B- | 62692 | 22-02-95 |
| | | IL-A- | 89649 | 31-07-95 |
| | | JP-A- | 2022285 | 25-01-90 |
| | | JP-B- | 8022865 | 06-03-96 |
| | | PT-B- | 90175 | 30-06-94 |
| | | US-A- | 5128331 | 07-07-92 |
| EP-A-440809 | 14-08-91 | JP-A- | 3077894 | 03-04-91 |
| | | AU-A- | 6168190 | 03-04-91 |
| | | CA-A- | 2037932 | 19-02-91 |
| | | WO-A- | 9102737 | 07-03-91 |
| | | US-A- | 5153183 | 06-10-92 |